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Evaluating the sensitivity, reproducibility and flexibility of a method to test hard shell capsules intended for use in dry powder inhalers



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ABSTRACT

Pharmaceutical tests for hard shell capsules are designed for orally administered capsules. The use of capsules in dry powder inhalers is widespread and increasing and therefore more appropriate tests are required to ensure quality and determine if these capsules are fit for purpose. This study aims to determine the flexibility, reproducibility and sensitivity of a quantitative method that is designed to evaluate the puncture characteristics of different capsule shell formulations under different climatic conditions. A puncture testing method was used to generate force displacement curves for five capsule formulations that were stored and tested at two different temperatures (5 °C and 19 °C). Force-displacement puncture profiles were reproducible for individual capsule shell formulations. The methodology was able to discriminate between capsules produced using different primary materials i.e. gelatin versus hypromellose, as well as more minor changes to capsule formulation i.e. different material grades and excipients. Reduced temperature increased the forces required for capsule puncture however further work is required to confirm its significance. Results indicate the method provides a reproducible and sensitive means of evaluating capsule puncture. Future studies should validate the methodology at different test sites, using different operators and with different capsule shell formulations.

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1. Introduction

Dry powder inhalers (DPIs) are used to deliver micronized drugs to the lungs, typically to treat pulmonary disease (Atkins 2005; Pavkov et al., 2010). In some DPIs hard shell capsules are used as a single-dose container for the drug in association with a carrier excipient. To facilitate pulmonary drug delivery from such devices the capsule wall is either punctured with a set of sharpened pins or cut by a blade. The powdered drug is then released from the capsule and entrained into the airflow upon inspiration by the user (Jones 2003). DPIs are established delivery systems that have formed the basis of many highly successful drug products for the treatment of asthma and COPD. In recent years there has also been an increase in new capsule products for use in DPIs.

A hard shell capsule consists of two open-ended cylinders, a cap and body, that fit one inside the other (Jones, 2004). The capsule

manufacturing process typically involves sourcing raw materials, preparing a solution of the capsule shell formulation, dipping standard rod-shaped-moulds into this solution, drying the films formed, removing them from the moulds and cutting the dried capsule shells to the correct length before joining the capsule body and cap together. Pharmaceutical materials used to manufacture DPI capsules are typically based on either gelatin or hydroxypropyl methylcellulose (HPMC). Gelatin, which is commonly derived from collagen that has been extracted from bovine bone and skin, has been used by the pharmaceutical industry to manufacture hard shell capsules for more than a century. The properties of this biological material are governed, in part, by the source of the raw material and the processing steps that are used. Under 'normal' storage conditions gelatin capsules have a water content of 13.0–16.0%. This water acts as a plasticiser in gelatin films and therefore in low humidity conditions gelatin capsules become brittle and prone to breakage on transport or during use (Jones 2003; Nagata 2002). HPMC capsules are used as an alternative to gelatin as they offer particular advantages: they are made from a plant-derived material, the capsule shells have a significantly lower moisture content, 4.0–6.0%, maintain appropriate physical properties when

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exposed to low humidities and provide a suitable container for moisture-labile compounds (Nagata 2002; Ogura et al., 1998).

Hard shell capsules were originally developed for oral dosage forms and the Pharmacopoeial tests are for filled capsules and not for empty ones. None are designed to evaluate the mechanical properties of capsule shells, a key parameter when the aim is to introduce an inhaler pin into the capsule to create a hole for powder emission. There is therefore a need to establish more predictive tests for capsule performance in DPIs that are robust enough to be used for quality assurance purposes and yet sensitive enough to detect minor changes in capsule properties. Our group have recently developed a methodology that can be used to evaluate the puncture performance of capsules that are to be used in DPIs (Torrìsi et al., 2013), however, further work is required to determine the reproducibility, sensitivity and flexibility of the method.

Patients do not always appreciate the importance of correct storage (Renswoow et al., 2010) or are unable to adhere to storage instructions. However, DPIs are used in countries and regions, with very different climatic conditions, and therefore filled capsules, for use in DPIs, are often packaged in blisters. Blister packing ensures that under adverse storage conditions capsules are offered some protection from moisture gain or loss. DPIs are used by patients in many different climatic conditions, for example in northern climes during winter they will be used at lower temperatures. The potential use of biologics in DPIs may also necessitate storage in the refrigerator. However, little is known about the influence of temperature on the performance of capsules in DPIs. This study uses a previously reported method (Torrìsi et al., 2013) to characterise the puncture of five different capsule formulations, using a pin from a DPI, at two different time points and at two different temperatures (5 °C and 19 °C). The aim is therefore to establish the reproducibility, sensitivity and flexibility of the method as a means to control the quality of empty capsule formulations, intended for use in DPIs, and potentially predict their performance under different climatic conditions. A secondary objective is to extend the previously described method to provide semi-quantitative characterisation of capsule punctures.

2. Materials and methods

2.1. Capsule conditioning at controlled temperature and humidity

Five different capsule shell formulations (labelled A–E; Table 1), provided by Qualicaps Europe, S.A.U. (Alcobendas, Spain), were conditioned either in a cold room (target temperature 5 °C) or in a temperature controlled room (target temperature 19 °C) for a minimum period of 7 days. All capsules were stored in desiccators over either a saturated solution of calcium chloride (Sigma–Aldrich, Poole, UK), to create a capsule moisture content in the lower part of the normal moisture specification limits (13–16% for gelatin and 4.5–6.5% for HPMC inhalation grade capsules), or magnesium nitrate (Sigma–Aldrich, Poole, UK), to create an environment that resulted in capsule formulations with moisture contents in the upper part of the normal range or slightly above. Capsules A–D consisted of a blend of HPMC, carrageenan

(a gelation aid) and potassium chloride (a network promoter). Capsule D was produced from HPMC that had been sourced from a different supplier than capsules A–C. Capsules A and D were colorless, capsule B contained titanium dioxide (and was therefore white) and capsule E contained FD&C yellow. The loss of water on drying (LOD) test (Council of Europe, 2005), was used to determine the moisture content of capsules both prior to and during the testing period.

2.2. Capsule puncture force measurements

The force of capsule puncture was measured and recorded using a Zwick materials testing machine (Herefordshire, UK), as previously described (Torrìsi et al., 2013). However, to enable testing to take place in different locations (a temperature controlled room and a cold room) the materials testing machine and accompanying hardware were mounted on a mobile heavy-duty trolley, which was re-positioned as necessary. Puncture tests were performed at a test speed of 10 mm/minute with an angular single metal pin from a RS01 2-pin inhaler (Plastiap S.p.A; Milan, Italy). This was attached via a chuck to the materials testing machine, which converts the force of puncture into a measurable electrical voltage. Tests were conducted multiple times ($N = 10$) at two different time points for each capsule type in order to evaluate test reproducibility.

2.3. Physical characterisation of punctured capsules

All punctured capsules from a single experimental set ($N = 10$) were mounted in a cap bushing from a capsule filling machine (Qualicaps Europe S.A.U) and inspected visually using an AmScope Stereo Inspection Microscope (CA, USA). Representative images were captured using the MU900 integrated digital camera ($\times 3.3$ – $\times 180$ zoom) within 30 min of capsule puncture. The shape of the capsule puncture was recorded as regular or irregular (Fig. 1), the presence or absence of a flap at the point of puncture was recorded and the cross sectional area of the puncture (Fig. 2) was measured, relative to the capsule cross sectional area, using ImageJ (NIH, USA) software.

2.4. Data processing and statistical analysis

Figures were generated and statistical analyses (unpaired two-tailed t -tests) were performed using Prism 5 for Mac OS X (GraphPad Software Inc., USA).

3. Results

3.1. Capsule conditioning

The mean average daily temperature in the temperature controlled room was 19 ± 0.19 °C and in the cold room was 4.8 ± 0.23 °C. The humidity in the temperature controlled room and cold room was $44.9 \pm 4.0\%$ and $81.8 \pm 5.2\%$ respectively, however, capsules were stored in desiccators in order to control their moisture content. Table 2 provides detail of the moisture

Table 1
The denominations and descriptions of the capsule shell formulations tested in this study.

Capsule label	Description	Principal material	Other excipients
A	Quali-V [®] -I transparent clear	HPMC	Titanium dioxide
B	Quali-V [®] -I opaque white	HPMC	
C	Quali-V [®] grade A transparent orange	HPMC	
D	Quali-V [®] grade B transparent clear	HPMC	
E	Hard transparent clear	Gelatin	

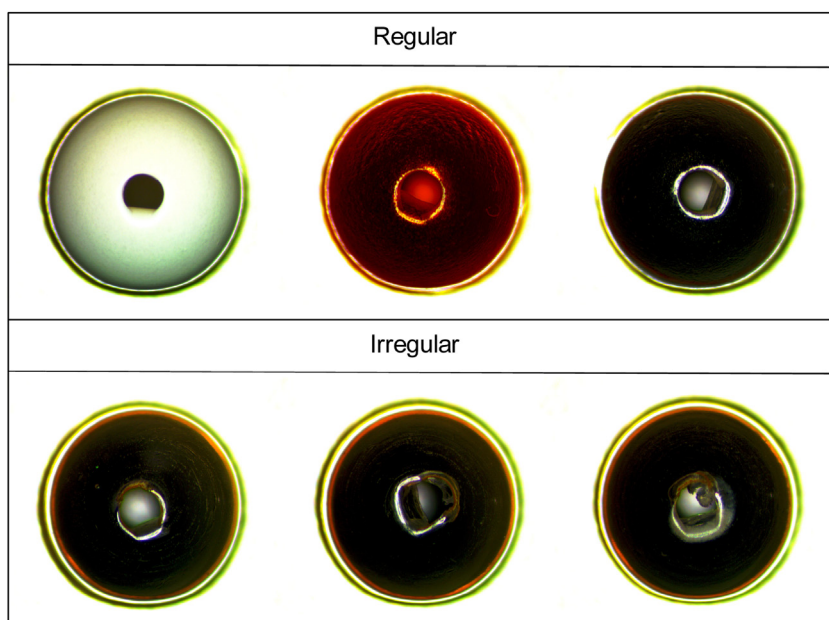


Fig. 1. Representative images of regular and irregular capsule puncture shapes in some of the capsules tested in this study.

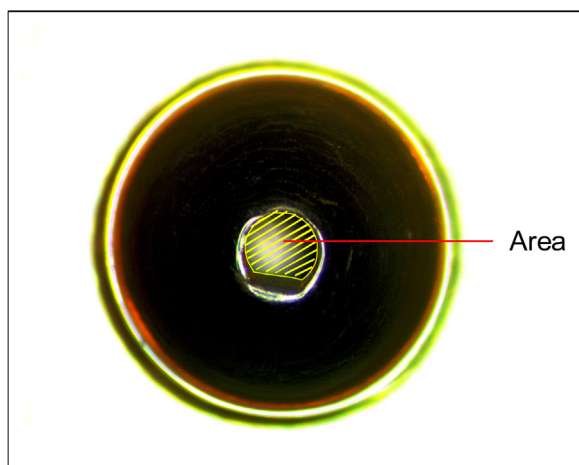


Fig. 2. A representative image to illustrate the area of capsule puncture that was recorded and quantified using ImageJ software.

content of capsules, calculated using LOD tests performed both before and during the puncture testing period. Generally, when stored at 19 °C over either CaCl_2 or $\text{Mg}(\text{NO}_3)_2$ the capsules moisture contents were at the lower and upper half of the normal moisture specification limits (4.5–6.5% for HPMC and 13.0–16.0% gelatin), respectively. At 5 °C the moisture content in desiccators was more difficult to control due to the higher atmospheric humidity. At 5 °C those conditioned over CaCl_2 were within the upper half of the

moisture specification and those conditioned over $\text{Mg}(\text{NO}_3)_2$ were slightly above the normal moisture specification.

3.2. Impact of formulation and climatic conditions on capsule puncture by a DPI pin

3.2.1. Evaluating the reproducibility of capsule puncture

The typical puncture force profile of a capsule has been characterised previously (Torrisi et al., 2013). The shape of the puncture profile (Fig. 3) and the maximum force recorded (i.e. the force required to puncture the capsule) (Table 3) is significantly different between capsule materials i.e. HPMC (Fig. 3A–D) versus gelatin (Fig. 3E). A double peak is clearly evident in Fig. 3E and is a signature of gelatin puncture, whereas this double peak is typically absent in HPMC puncture profiles (Fig. 3A–D). More minor changes to the HPMC capsule formulation also change the magnitude of the puncture force (Table 3), although the overall shape of the puncture profile appears less affected.

The reproducibility (intra-experimental variability) of the method is demonstrated by comparing individual profiles within a single set of data (Fig. 3A Set 1) and the repeatability of the test (inter-experimental variability) can be evaluated by comparing experiments conducted 8 days apart (Fig. 3A Set 1 versus Set 2). The intra- and inter-experimental reproducibility of the methodology, at both the studied temperatures, is exemplified by the puncture forces and associated standard deviations for capsule formulation A (Table 3). Other capsules, most notably capsule C, have more variable puncture forces within an experiment,

Table 2

The mean (\pm standard deviation) moisture content of capsule formulations A–E when stored at either 5 °C or 19 °C and over either CaCl_2 or $\text{Mg}(\text{NO}_3)_2$. Results are from ≥ 2 LOD tests conducted on different days.

Condition		Capsule type				
		A	B	C	D	E
19 °C	CaCl_2	4.38 \pm 0.39%	4.50 \pm 0.21%	4.85 \pm 0.11%	4.60 \pm 0.24%	13.22 \pm 0.37%
	$\text{Mg}(\text{NO}_3)_2$	6.64 \pm 0.23%	6.17 \pm 0.42%	6.51 \pm 0.38%	6.23 \pm 0.42%	14.58 \pm 0.32%
5 °C	CaCl_2	6.07 \pm 0.43%	5.90 \pm 0.33%	6.17 \pm 0.28%	5.82 \pm 0.18%	14.60 \pm 0.10%
	$\text{Mg}(\text{NO}_3)_2$	7.16 \pm 0.54%	6.95 \pm 0.30%	7.17 \pm 0.53%	7.06 \pm 0.16%	14.80 \pm 0.01%

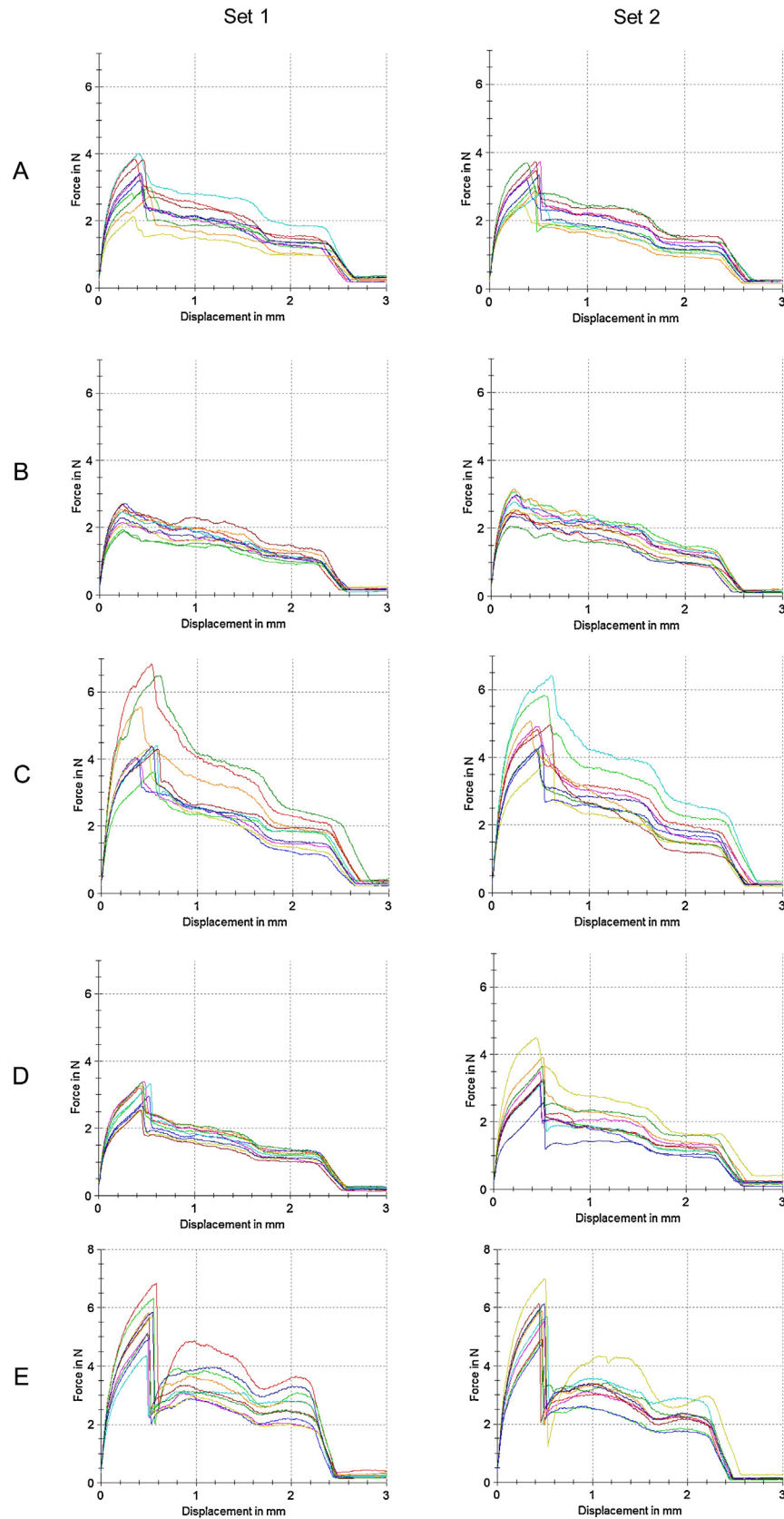


Fig. 3. The puncture profiles of 5 different capsule formulations (labelled A–E) stored and tested at 19 °C in CaCl₂. Data is presented in duplicate sets (set 2 is an experimental replicate of set 1, conducted 8 days later) to evaluate reproducibility. Force_{max} = force required for needle to penetrate capsule shell; dL at force_{max} = distance of needle displacement prior to penetration. *N* = 10 for each capsule type in each condition.

Table 3

The mean (\pm standard deviation) maximum force (F_{\max}) recorded upon penetration of capsule formulations A–E when stored at either 5 °C or 19 °C and over either CaCl_2 or $\text{Mg}(\text{NO}_3)_2$. Results of two experimental sets (each $N = 10$) are presented to demonstrate reproducibility of the methodology.

Condition		Experiment	Capsule formulation				
			A	B	C	D	E
19 °C	CaCl ₂	1	3.25 ± 0.57 N	2.34 ± 0.30 N	4.79 ± 1.10 N	3.03 ± 0.34 N	5.57 ± 0.72 N
		2	3.24 ± 0.43 N	2.69 ± 0.35 N	4.90 ± 0.75 N	3.40 ± 0.52 N	5.67 ± 0.70 N
	Mg(NO ₃) ₂	1	2.56 ± 0.34 N	2.21 ± 0.24 N	3.93 ± 1.09 N	2.81 ± 0.45 N	5.28 ± 0.93 N
		2	2.67 ± 0.28 N	2.40 ± 0.30 N	3.67 ± 0.58 N	2.99 ± 0.62 N	5.43 ± 0.75 N
5 °C	CaCl ₂	1	3.24 ± 0.80 N	2.56 ± 0.34 N	4.61 ± 0.59 N	3.62 ± 0.84 N	6.31 ± 0.96 N
		2	3.43 ± 0.51 N	2.67 ± 0.43 N	5.24 ± 2.50 N	3.71 ± 0.91 N	5.47 ± 0.60 N
	Mg(NO ₃) ₂	1	2.96 ± 0.37 N	2.51 ± 0.45 N	3.86 ± 0.57 N	2.66 ± 0.32 N	6.13 ± 0.80 N
		2	2.78 ± 0.46 N	2.23 ± 0.14 N	4.31 ± 0.79 N	2.85 ± 0.47 N	5.84 ± 0.89 N

exemplified by greater divergence in the puncture profiles (Fig. 3C) and greater standard deviation values (Table 3).

3.2.2. The influence of formulation on the force required to puncture capsules with a DPI pin

Fig. 4 combines data from the first and second set of experiments to summarise the differences in the puncture force required for the capsule shell formulations A to E when

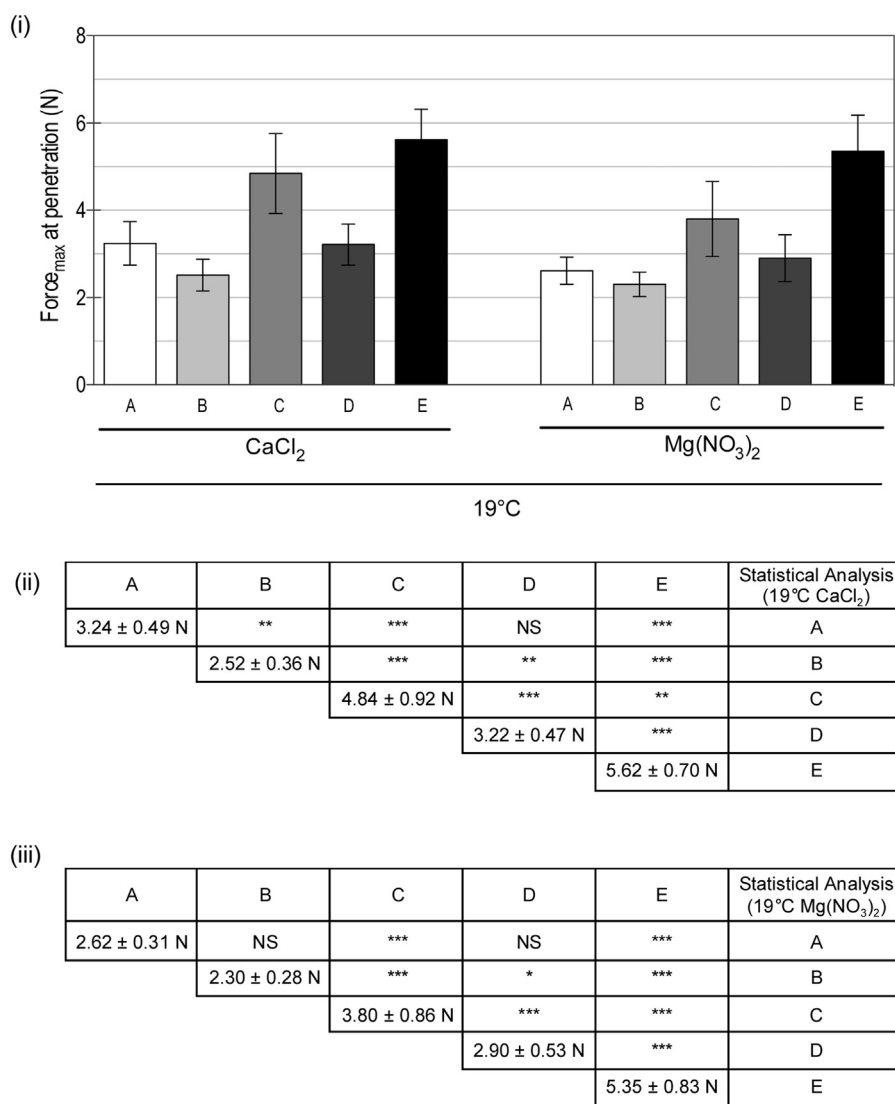


Fig. 4. The impact of **capsule shell formulation** on the force needed to puncture capsules with a DPI pin at 19 °C. (i) A comparison of the mean puncture force recorded for five capsule shell formulations (A–E) that are stored over CaCl_2 or $\text{Mg}(\text{NO}_3)_2$. Error bars represent standard deviation; $N = 20$. Statistical differences in the puncture force of the five different capsule formulations (A–E) with the DPI pin, conditioned either over CaCl_2 (ii) or $\text{Mg}(\text{NO}_3)_2$ (iii) were determined using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. NS denotes a $p > 0.05$; *** denotes $p < 0.001$, ** denotes $p < 0.01$ and * denotes $p < 0.05$ ($N = 20$).

conditioned at 19 °C and at two different humidities. The puncture forces required for the gelatin capsule (capsule E) are significantly different from all HPMC formulations (capsule A–D) at both humidities ($p < 0.001$ in all cases except with capsule C stored over CaCl_2) (Fig. 4). There are also significant differences ($p < 0.001$) between the puncture force for capsule C and all other HPMC capsule formulations (Capsules A, B and D) at both humidities. The differences in puncture force between capsules A, B and D were less significant and in some cases there was no statistical difference, most notably in the case of capsules A and D, which were not significantly different at either of the tested humidities (Fig. 4).

3.2.3. The influence of storage temperature on the force required to puncture capsules with a DPI pin

Capsule shell formulations conditioned over CaCl_2 i.e. the lower moisture specification tested, required greater forces to facilitate puncture compared to those conditioned over $\text{Mg}(\text{NO}_3)_2$ (Fig. 5i). This difference is statistically significant in HPMC capsule formulations A, B and C at 19 °C, and capsule formulations A, B and D stored at 5 °C ($p \leq 0.05$). The differences between capsule puncture forces at the two studied temperatures (5 °C and 19 °C) were less predictable (Fig. 5ii). There was a trend towards higher puncture forces being required at the lower temperature. This difference was not statistically significant except in the cases of the puncture forces required for capsule D when conditioned over CaCl_2 and capsule formulations A and E when stored over $\text{Mg}(\text{NO}_3)_2$ ($p < 0.05$). Generally, temperature (Fig. 5ii) generally had

Table 4

A summary of the percentage of capsules that displayed a 'regular' shaped puncture following removal of the angular DPI pin from punctured capsules ($n = 10$).

Condition		Capsule type				
		A	B	C	D	E
19 °C	CaCl_2	100%	100%	100%	100%	40%
	$\text{Mg}(\text{NO}_3)_2$	100%	100%	100%	100%	80%
5 °C	CaCl_2	100%	100%	100%	100%	50%
	$\text{Mg}(\text{NO}_3)_2$	100%	100%	100%	100%	60%

less impact on the puncture force than the moisture content (Fig. 5i).

3.2.4. The influence of formulation and storage conditions on the puncture area created in a capsule by a DPI pin

The shape (Table 4) and area (Fig. 6) of capsule punctures provided qualitative data related to the puncturing event. At both 19 °C and 5 °C the shape of the puncture created in the HPMC capsule formulations (A–D) was considered to be "regular" (Fig. 1) on all occasions, whilst between 20% and 60% of the gelatin capsules had irregular puncture shapes, depending on the capsule storage conditions (Table 4). A greater percentage of gelatin capsules with lower moisture contents (stored over CaCl_2) had irregular shaped punctures compared to those capsules with higher moisture contents (stored over $\text{Mg}(\text{NO}_3)_2$). Following puncturing a flap was visible in all HPMC and gelatin capsules.

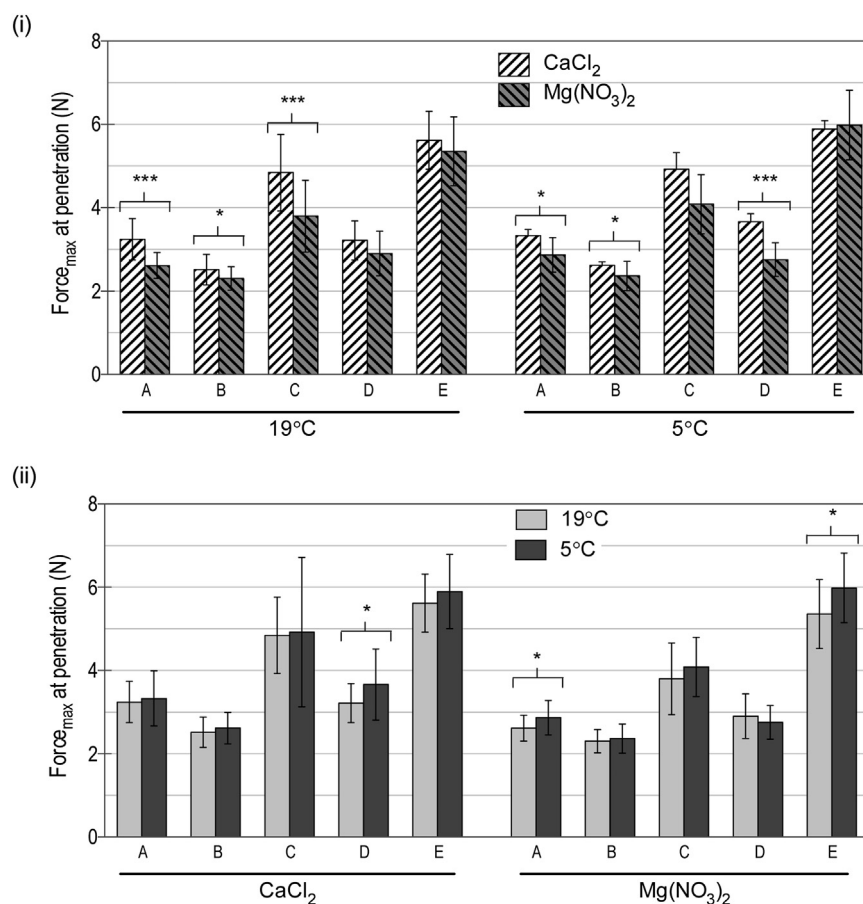


Fig. 5. The impact of storage conditions on the force needed to puncture capsules with a DPI pin. A comparison of the mean puncture force recorded for five capsule formulations (A–E) that are either over (i) CaCl_2 or $\text{Mg}(\text{NO}_3)_2$ and (ii) at 19 °C and 5 °C. An independent sample two-tailed t -test was used to calculate significant differences in the compared groups, which are denoted by * ($p < 0.05$) or *** ($p < 0.001$); error bars represent standard deviation; $N = 20$.

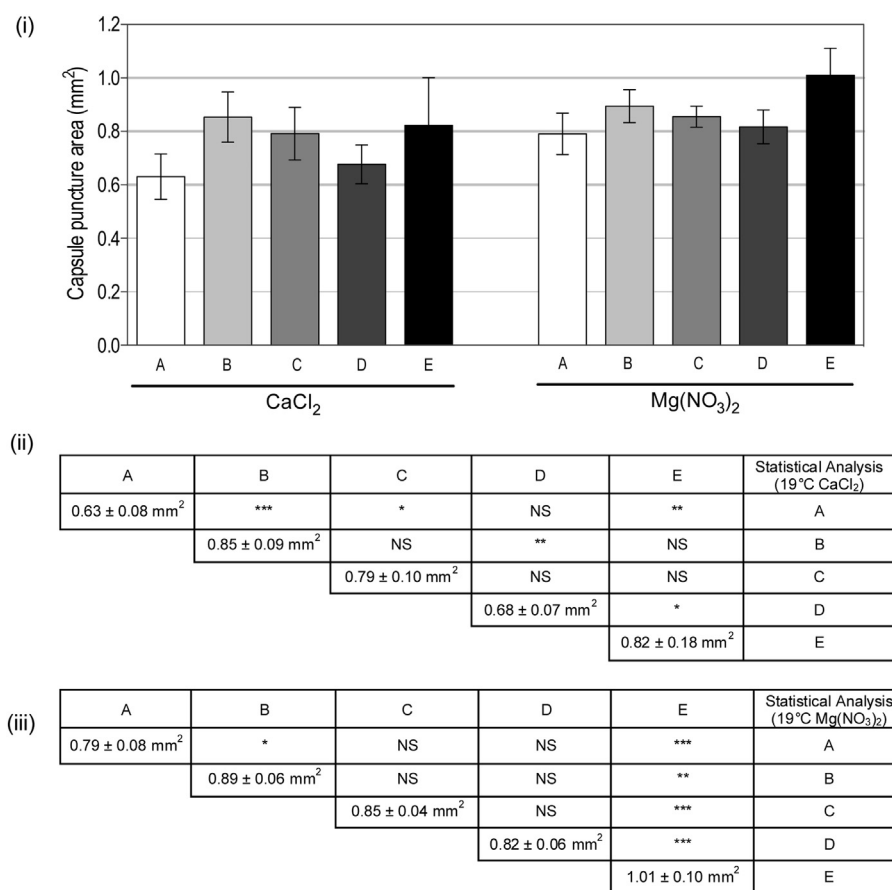


Fig. 6. The impact of capsule shell formulation on the puncture area created by a DPI pin at 19 °C. (i) A comparison of the mean puncture area recorded for five capsule shell formulations (A–E) that are stored over CaCl₂ or Mg(NO₃)₂. Error bars represent standard deviation; *N* = 10. Statistical differences in the puncture area of the five different capsule formulations (A–E), conditioned either over CaCl₂ (ii) or Mg(NO₃)₂ (iii) were determined using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. NS denotes *p* > 0.05; *** denotes *p* < 0.001, ** denotes *p* < 0.01 and * denotes a *p* < 0.05 (*N* = 10).

At 19 °C the cross sectional area of the punctures in HPMC capsules stored over Mg(NO₃)₂ ranged from 0.79 ± 0.08 mm² for Capsule A to 0.89 ± 0.09 mm² for Capsule B. This difference was statistically different (*p* < 0.05), although it should be noted that the puncture areas for other HPMC capsules stored over Mg(NO₃)₂ were not statistically different (Fig. 6ii). At this humidity, differences between the puncture area of all HPMC capsule formulations and the gelatin formulation were clearly evident, with all four HPMC capsules possessing notably smaller puncture areas than their gelatin counterpart. At the lower humidity (capsules stored over CaCl₂) the puncture areas were generally smaller than the higher humidity (Figs. 6i and 7 ii), although this was not always statistically significant. Differences between the gelatin and the HPMC formulations were less obvious. At the lower moisture content results were more comparable to the puncture force data (Fig. 4ii), whereby formulations A and D performed comparably and were significantly different to gelatin capsules (capsule E).

In general, capsules stored and tested in a temperature controlled room (19 °C) produced significantly larger capsule puncture areas compared to those capsules stored and tested at the lower temperature of 5 °C (Fig. 7i). This difference was statistically significant (*p* < 0.01) in all of the tested formulations except gelatin capsules (capsule E) stored over CaCl₂. Capsules with higher moisture content (stored over Mg(NO₃)₂) appeared to produce slightly larger puncture areas compared to those capsules with lower moisture content (stored over CaCl₂), although these differences were not always statistically significant (Fig. 7ii).

4. Discussion

The performance of hard shell capsules in a DPI is affected by numerous factors including the capsule formulation (Coates et al., 2005), the DPI design (Martinelli et al., 2015; Shur et al., 2012) and use of the device within the clinical environment (Islam and Cleary, 2012). In this study a novel capsule puncture method, previously described by Torrisi et al. (2013), was extended to evaluate whether the methodology was sensitive enough to detect minor changes in capsule formulation, robust enough to provide reproducible data at two different time points and flexible enough to evaluate capsule puncture at low temperatures.

The puncture forces recorded for a specific formulation, stored in controlled conditions, were reproducible within experiments (*N* = 10) and were associated with small standard deviations (Table 3). The shapes of the puncture profiles were also comparable (Fig. 3). The reproducibility of the puncture test data obtained at two different time points further exemplifies this and indicates that the method is robust, both at the intra- and inter-experimental levels, and therefore appropriate as a means to investigate how capsule formulation and storage parameters can influence the properties of hard shell capsules.

Results support previous observations, Torrisi et al. (2013), in which the method was able to clearly discriminate between capsules produced using different primary materials i.e. HPMC and gelatin capsules. However, data presented in this study also indicates that the method is able to detect more minor changes to

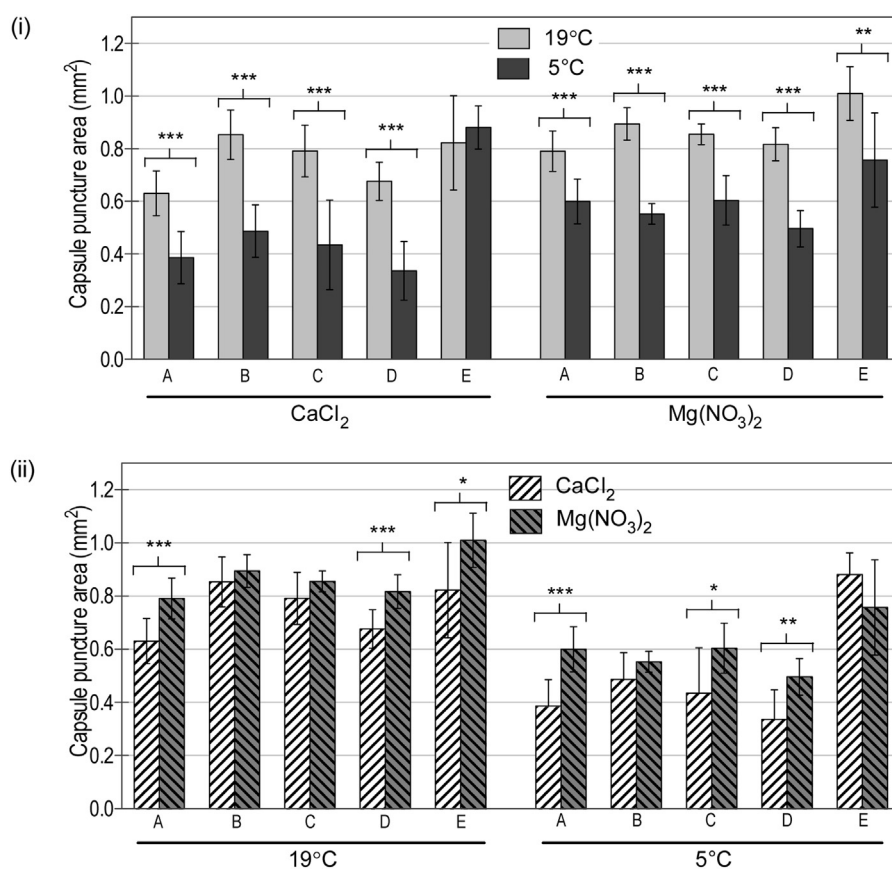


Fig. 7. The impact of storage conditions on the puncture area created by a DPI pin that is inserted into the capsule at a known speed. A comparison of the mean puncture force recorded for five capsule formulations (A–E) that are stored at 19 °C and 5 °C (i) and either over CaCl₂ or Mg(NO₃)₂ (ii). An independent sample two-tailed *t*-test was used to calculate significant differences in the compared groups, which are denoted by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$); error bars represent standard deviation; $N = 10$.

the capsule shell formulation, such as the use of colorants and other excipients. Changes to the raw HPMC material (Capsule D) has had little effect on the capsules puncturing properties, however results for capsule C indicate that small changes to the formulation of a capsule can have significant effects in terms of the puncture force required and also the variability of this puncture performance (Fig. 3). This method is therefore highly relevant to those involved in the formulation and quality control of hard shell capsules, whereby changes to capsule materials, formulations or their method of manufacture could impact their performance. Current pharmacopoeial tests for capsule are for filled products, e.g. disintegration and dissolution tests, but these tests were developed for oral capsules. A specific puncture force and its associated puncture profile may provide a standard measure that can be used to control the quality of capsule shells intended for use in DPIs. Our method (Torrissi et al. in 2013) therefore has the potential to provide the sensitivity and reproducibility that would be required for a standardised assay of capsule puncture.

Previous studies have illustrated the effect of humidity on the water content of capsules and their resulting performance in DPIs (Birchall et al., 2008). Significant differences in the force required to puncture capsules stored at 'normal' and 'low' humidities have also been identified previously (Birchall et al., 2008; Torrissi et al., 2013), with capsules with lower moisture contents requiring a higher force for puncture compared to capsules with higher moisture contents. These studies illustrate that uncontrolled storage conditions, with respect to humidity, can impact on capsule puncture and potentially performance. Capsules used in

this study were conditioned to be within their moisture specifications, at the lower and upper ends of the 'normal range'. Although the difference in moisture content was minimal, and acceptable with respect to clinical use, capsule puncture forces were higher at the lower moisture content for all formulations tested at 19 °C (Fig. 5i). Not all of these differences were statistically significant but the data is consistent with observations in previous studies as HPMC and gelatin capsules with lower moisture contents are less pliable and thus are more resistant to puncture (Birchall et al., 2008; Torrissi et al., 2013). This indicates that the puncture testing method is sensitive enough to detect changes in capsule puncture properties, which may result from storage at slightly different relative humidities. However, although the data exemplifies the sensitivity of the method and its potential in the quality control of capsules, it does not predict whether such changes will have impact on clinical utility.

In general, greater puncture forces were recorded for capsules stored and tested in the cold (5 °C) room compared to the air-conditioned (19 °C) room, however in many cases these differences were not statistically significant (Fig. 5ii). It may therefore be concluded that reduced temperature has little effect on the forces required to puncture capsules. However, the capsule moisture content of each capsule shell formulation was higher at the lower temperature (Table 2) and this is a confounding factor. The moisture content of capsules stored at the higher humidity (stored over Mg(NO₃)₂) increased by a mean average 0.70% for the HPMC capsule formulations (Capsules A–D) and 0.22% for the gelatin capsule formulation (Capsule E) when the storage temperature

was reduced from 19 °C to 5 °C. However, at the lower humidity (capsules stored over CaCl_2) the reduction in temperature resulted in a mean average 1.41% increase in the moisture content of HPMC capsule formulations and a 1.38% moisture content increase for the gelatin capsule formulation (Table 2). Increased capsule moisture content reduces the force required to puncture capsules (Fig. 5i). Therefore whilst the data indicates a trend towards lower puncture forces being required at the lower temperature, this is likely to be offset by a simultaneous rise in the moisture content of the capsules. Data for capsules stored over $(\text{MgNO}_3)_2$, where moisture content was more comparable, may be considered to be more representative of the impact of temperature alone on puncture force but the high sensitivity of the method means that the influence of the increased humidity at lower temperatures cannot be discounted. The influence of low temperature on both the capsule moisture content and the puncture properties of capsules should therefore be considered during the manufacture, packaging and clinical use of capsules.

The shape and area of the capsule puncture is another indicator of quality of inhalation grade capsules and also potentially influences drug powder release from the capsule. In all conditions tested HPMC capsules produced regular shaped punctures with flaps attached, whilst gelatin capsules (capsule E) had a proportion of irregular shaped punctures. This, and other studies have indicated that HPMC inhalation grade capsules are more flexible than gelatin (Kuentz et al., 2006; Podczeczek, 2002; Torrisi et al., 2013) and all HPMC formulations tested in this study produced more reproducible puncture shapes (Table 4). However, further work is needed to confirm whether irregular puncture shapes impact on the clinical performance of the capsule, i.e. dose emission and pulmonary deposition.

Differences in the puncture areas between capsule formulations were less predictable. However, results indicate that capsule puncture area is significantly affected by temperature, whereby capsules stored and tested at 5 °C had much smaller areas compared to those tested at 19 °C. This difference was significant and consistent for all capsule formulations tested. Whilst this is an interesting observation, it is difficult to disentangle the impact of temperature and humidity, as previously discussed. This particular finding warrants further investigation as major differences in the size of the orifice created in a capsule may impact on DPI performance and the resulting clinical outcome. However, it is important to stress that the physical characterisation of capsule puncture area is a semi-quantitative method that is constrained by the imaging technique i.e. a microscope captures a two-dimensional image of a three-dimensional event. Thus this technique has some limitations for routine quality control use, but does provide a good qualitative indication of how storage conditions affect capsule puncture area. Future studies should investigate the potential of imaging systems that can provide three dimensional images i.e. data in the x, y and z plane, in order to better characterise the orifice, and the associated 'flap', that is created in a capsule during puncture by a DPI. This would provide an estimate of the volume of the aperture through which powdered drug must flow in order to be aerosolised and inspired by a patient.

Current quality assurance testing for capsules uses tests that are developed for orally administered dosage forms. Therefore, it is important that a robust method for testing capsules that are used in DPI products is developed for routine quality assurance during capsule shell manufacture. The consistency, reproducibility and flexibility of the puncture force testing methodology described in this study has significance for those developing and manufacturing hard shell capsules. Capsule manufacturers could adopt this rapid and robust testing methodology to evaluate how manufacturing variations, such as different raw materials, different manufacturing sites and changes in storage, will affect the puncture quality of the

capsules produced. This study has also illustrated the flexibility of the method, conducting tests at two different temperatures. However this could be taken further, as the method could be easily adapted to use different DPI pins and different insertion speeds to more closely mimic a specific DPI. In addition to puncture performance the methodology has the potential to provide a predictor of DPI performance, however further work is needed to correlate capsule puncture with powder aerosolization and subsequent deposition.

5. Conclusion

This study demonstrates the flexibility, reproducibility and sensitivity of a methodology that can be used to determine differences in the puncture performance of different capsule shell formulations stored at different humidities and at different temperatures. This simple and rapid laboratory test provides a valuable tool for the quality assurance of commercial capsule shell formulations, intended for use in DPIs, made from different materials or using different manufacturing methods. Further work is now required to provide three-dimensional characterisation of the orifice created in a capsule by a DPI and to determine how features of capsule puncture correlate with subsequent DPI performance.

Whilst further work is needed to ascertain the impact of temperature alone on puncture performance, results suggest that capsule storage in a low temperature (5 °C) environment necessitates slightly greater forces to facilitate capsule puncture and results in orifices that are smaller than those created at room temperature. This may have implications for DPIs used in colder climates. Further work needs to be conducted to better understand the relationship between temperature, humidity and the force required for capsule puncture.

Declaration of interest

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